



# Facilitating the gut brain axis by probiotic bacteria to modulate neuroimmune response on lead exposed zebra fish models

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**ABSTRACT.** Unravelling the efficacy of gut biome has a major impact on health. An unbalanced microbiome composition is linked to many common illnesses such as gut dysbiosis, mental deformities and immunological imbalance. An optimistic influence on the gut biome can be made by consuming probiotics. This would stimulate neuroprotection and immunomodulation intended by heavy metals pollution. Lead is a major source of neurotoxin that can induce neural deformities. *Lactobacillus* species isolated from curd were characterized to confirm its specificity. Zebra fish was reared at standard conditions and preclinical assessment on the intensity of induced neurotoxin lead was performed. The embryo toxic assay, immunomodulation effects and animal behavioural models endorsed the consequence of neurotoxicity. Different concentrations of bacterial isolate with standard antidepressant was considered for analysing the vigour of toxicity and its influence on cognitive behaviour by novel tank diving method. The restraint in the animal behaviour was also conferred by all the test samples with a decreased bottom dwelling time which was authenticated with haematology and histopathological studies. The alterations in morphology of the lymphocytes were balanced by the treated test samples. This study paves a twofold potential of probiotic as neuroprotectant and immune modulator against heavy metal toxicity.

**Keywords:** heavy metals; neurotoxicity; *Lactobacillus* species; probiotics; neuroprotection; immunomodulation; Zebra fish.

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## Introduction

Mental ailment profoundly invades the personal existence of human being and there are varied reasons for its commencement. The World Mental Health Survey Initiative (WMHS) which has coordinated mental health surveys across 28 countries has reported mental disorders (depression) as global burden of diseases. In 2010, it has established global burden of disease project (GBD) that focused on quantitative approaches designed to integrate find solutions towards psychiatric epidemiological studies (Steel et al., 2014). The consequences of damage to the nervous system can lead to mental deformities such as convulsions, paralysis, dementia, coma, early developmental distortion and incoordination. A minor level of neural damage can impair reasoning ability, loss of memory and lack of motor coordination which can intrude our daily activities.

Neurotoxins are chemical, physical or biological agents that cause adverse effects in nervous system. In recent years heavy metal toxicity has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications. Lead (Pb) is a heavy metal that is naturally occurring in earth's crust and is prone to induce toxicity, genotoxicity, and carcinogenicity (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). The World Health Organization has identified lead as one among ten topmost chemicals with a threat in public health concern. Further, it has been found in at least 1,272 of the 1,684 National Priority List (NPL) sites identified by EPA (Environmental Protection Agency) and demands stringent management (Glenn, 2002; Wynne, 2008).

Lead is used as an active ingredient in many products such as car batteries, paints, ceramics, cosmetics and shielding material with a wide industrial perspective (National Institute of Environmental Health Sciences, 2008). There is a high incidence of human exposure to (Pb) in the environment and is considered as a hazardous toxin.

Conversely, it is one of the toughest metal that can be strongly absorbed to soil with an enormous durability. Brain is a sensitive organ that is intensely susceptible to the neurotoxic effects of (Pb), which has been reported to induce damage to the nervous system. The neural homeostasis mechanism is disrupted abruptly by the mimicking ability of (Pb) that inhibits the action of calcium which alters the behaviour of endothelial cells and weakens the blood brain barrier (Sanders, Liu, Buchner, & Tchounwou, 2009).

Gut is an imperative organ that dwells diverse and vivacious population of micro-organisms known to have an association with brain thereby establishing a novel gut neural axis. Gut microbiomes perform vital physiological functions such as assimilation of nutrients, stimulation of immune system and regulation of cognitive function (Kelly et al., 2015). The metabolite of the microbiome reaches the brain through the blood brain barrier (BBB) and can cause changes in the neuronal membranes (Walter & Carraretto, 2016). Subsequently, these gut bacteria termed as psychomicrobiotics are able to produce active metabolites that have normalized anxiety like behaviour and itself acts as a therapeutic agent. *Lactobacillus* is one of the diverse and phylogenetically heterogeneous orders of lactic acid producing bacteria that claims to be an efficient probiotic (George et al., 2018).

Consumption of probiotic can modulate the brain-gut-micro biota axis to have a beneficial effect on mood, anxiety and cognition. Moreover, the adhesion of lactobacilli to intestinal tract interacts with toll-like receptors and stimulates the host's immune response which contributes anti-inflammatory effects (Desbonnet, Garrett, Clarke, Bienenstock, & Dinan, 2008). Therefore, the addition of *Lactobacillus* probiotics flourishes the gut-micro biota and act as a beneficial adjuvant therapeutic effect on the treatment of psychological disorders (Halder & Mandal, 2015). Zebra fish (*Danio rerio*) has become a powerful model organism for research into profound areas of vertebrate genetics, drug development, regeneration, and toxicology studies. They are also becoming increasingly popular in neuroscience research that includes in the screening of psychotropic drugs (Bencan, Sledge, & Levin, 2009; Norton, 2013). This study is devised to isolate a competent probiotic strain from fermented milk sample that can authenticate its therapeutic effect on animal model (Zebra fish) against the influence of lead as a neurotoxicant.

## Material and methods

### Chemicals

All chemicals and dyes used in this study were of analytical grade, purchased from Sigma, India. The bacteriological media were obtained from Hi Media Laboratories Pvt. Ltd., India.

### Isolation and characterization of *Lactobacillus* from fermented milk samples

The milk sample was collected from country breed cows housed in a local farm located in R. R. Nagar, Virudhunagar, Tamilnadu India. Immediately after collection the sample was stored aseptically in low temperature (40C) for the isolation of *Lactobacillus*. The strain was isolated by the process of serial dilution and microbial plating techniques. The milk sample was plated on nutrient agar plates containing 20% of raw milk. The plates were incubated at 37°C for 24 hours. The colonies were isolated and maintained on nutrient agar slants. Identification of the isolated bacteria as *Lactobacillus* species was performed according to their morphological, cultural, and physiological and biochemical characteristics by the procedures as described in Bergey's Manual of Systematic Bacteriology (Bergey & Holt, 2000). The tests carried out were Gram staining, motility test, H<sub>2</sub>S formation, starch hydrolysis, sugar fermentation profile, gelatin hydrolysis, production of catalase, nitrate reduction, endospore and acid production test.

### Assessing probiotic property

The probiotic properties of the isolated lactobacilli was determined by analysing its resistance to sodium chloride and low-pH. The tests were performed in accordance to the protocol of Liong and Shah (2005) with minor modifications as, cited by Chowdhury et al. (2012). The bacterial isolate was grown (for 24h at 37°C), in nutrient broth with sodium chloride supplementation with a run of concentration (2,3,4,5,6,6.5,7,7.5 and 8%,) and then sub cultured on agar (24h at 37°C). The resistance of the bacterial isolate to low pH was analysed by suspending the overnight cultures (h) viability. The tests were conducted under atmospheric carbon dioxide, and were 18 replicated twice.

### Rearing and Maintenance of parental fish

Adult zebra fishes were obtained from a commercial dealer and 10-15 fishes were kept in 5 litre acrylic tank with the following conditions; 28.5°C, with a 14 10h<sup>-1</sup> light dark<sup>-1</sup> cycle. All protocols were reviewed and approved in accordance with by the Institutional Ethical Committee guideline No. 203 (Fish, acute toxicity test) and No. 212 (Fish, short-term toxicity test on embryo and sac-fry stages). The zebra fish were 2-3 months old, weighed 0.5 ± 0.2 g and their mean length was 30 ± 4 mm. The fishes were fed three times per day, with commercially available dry fish food. Embryos were obtained from natural spawning that was induced at the morning by turning on the light. Collection of embryos was completed within 30 min.

### Determination of LC50 value for the test sample

*Lactobacillus* strain (sample) is tested in a series of 8 (5, 10, 25, 50, 75, 100, 500 and 1000 ug) different concentrations constituting an approximate geometric progression. The experiment is conducted in glass aquaria with 30 litres of water capacity. The embryos are divided into 9 groups (including one control group), each containing 30 nos. The tests are carried out using a semi-static method with solution replacement after 24 hours. During the tests, the water temperature, pH value, dissolved oxygen concentration in each of the test tanks, fish behaviour and mortality rate are recorded individually in each test container at exposure times of 24, 48, 72 and 96 hours on daily basis. The test groups were kept under the 12-h/12-h light/dark cycle; the dissolved oxygen concentrations did not fall below 60% (89-100%); and the pH ranged between 6.10 and 7.92. *Lactobacillus* strain is used for the preparation of stock solutions that is diluted as desired. Embryos were exposed for 96 hours, separately, against different 5 concentrations of sample with an increment of 1 mg L<sup>-1</sup> for low to high concentrations, respectively. Finney's Probit analysis method is used to calculate the 96-hr LC50 and the value were plotted in MS- Excel Software.

### Studies on embryonic degeneration and developmental changes (test/standard antidepressant

Embryonic degeneration studies were performed on eggs of *Danio rerio* in accordance to the protocol of Meyers (2018). A series of varied concentrations of 6 different test samples as mentioned in the Table 1 was taken for experimentation.

**Table 1.** Protocol design representing groups of animals and dose of samples administered.

Groups	Treatment	Dose (µg mL <sup>-1</sup> day <sup>-1</sup> )	Dose (Volume mL)	Routes of Administration
I	Devoid of treatment (with Pb)	0.29	1 mL	mixed in water
II	Normal	Saline	1 mL	Oral
III	Pb + strain (high)	0.29 + 100	1 mL	Oral
IV	Pb+ strain (low)	0.29 + 50	1 mL	Oral
V	Pb + setraline (high)	0.29 + 1	1 mL	Oral
VI	Pb + setraline (low)	0.29 + 0.1	1 mL	Oral

Two LC50 tested concentration of *lactobacillus* strain were considered as high and low dosage. Dilution of the stock solution is used for the preparation of the concentrations tested. 30 fertilized eggs in a Petri dish are tested at each concentration and in one control. The volume of liquid is 20 mL in each Petri dish. The eggs are placed in the Petri dishes within 8 hours after fertilization. The test is terminated after hatching and the absorption of the yolk sack in all individuals in the control dish. Early life stage parameters such as egg and embryo mortality, gastrulation, somite formation, movement and tail detachment, pigmentation, heart rate, and hatching success are noted. During the test, the number of dead embryos in individual concentration was recorded.

### Behavioural studies on depression induced fish models

#### Novel tank test:

Novel tank test is a sensitive and efficient behavioural assay (Levin, Bencan, & Cerutti, 2007) to study the anxiety-like behaviour of the fish (bi-directionally) altered by drugs affecting the gamma amino butyric acid, mono aminergic, cholinergic, glutamatergic and opioidergic system. It assesses behavioural indices of anxiety (including reduced exploration, increased freezing behaviour and erratic movement). In a novel

tank, Zebra fish dwell in the bottom of the tank initially and then increase their swimming exploration to higher levels over time. The specificity of the diving effect was corroborated with a novel vs. non-novel test tank. The novel tank diving response of Zebra fish is tried when given a depressant drug. This protocol is an easy, economical and effective alternative to other methods of quantifying stress responses in Zebra fish. Fish anxiety-like behaviour can be either reduced or aggregated depending on stress or drug exposure, with cortisol levels generally expected to parallel anxiety behaviours. This protocol can be accomplished over the course of 3 days, with an adaptable testing duration depending on the number of fish used.

### **Haematological assay and lymphocyte counting**

Blood was collected by puncturing the tails of the fishes (respective groups) exposed in tricaine solution with heparin zed tips. The collected blood is suspended with phosphate-buffer saline (50  $\mu$ L). Blood welling up from this incision was rapidly collected by a micropipette tip and used in preparing blood smears. Blood smears were prepared on glass slides and were placed in a fixative solution for 15 seconds and air dried. Slides were then stained with Wright Giemsa stain and examined under oil immersion by light microscopy. Identification of zebra fish peripheral blood cells was based on the studies of Rowley et al. (1997). Differential counts of red cells, leucocytes and thrombocytes were performed by the differentiation based on morphological appearance.

### **Histopathological examination**

The specific areas of brain (cerebellum) and skeletal tissues were fixed in par formaldehyde, dehydrated through a graded series of ethanol, cleared in xylene and embedded in section of 7  $\mu$ m thickness were prepared from paraffin blocks using a rotary microtome and then stained with haematoxylin-eosin. Histopathological changes were examined under microscope.

## **Results**

### **Isolation and identification of *Lactobacillus* sp.**

The bacteria were isolated from fermented milk sample. These isolates were grown in Nutrient Agar Medium at pH 6.5. They were identified as *Lactobacilli* by observing their colony morphology, cultural, physiological and biochemical characterization. Colony characteristics of *Lactobacilli* isolates were studied by picking-up a single well isolated colony aseptically and transferred to selective medium to observe the growth pattern of isolates on nutrient agar medium. Colonies appeared small, irregular, round shape, creamy white colored, circular, low convex with entire margin were regarded as belonging to the genus *Lactobacillus* (Table 2).

### **Biochemical characterization and probiotic assessment**

The strains were phenotypically characterized on the basis of their morphological, cultural, and biochemical features. Gram's staining of the bacterial culture revealed that they were gram positive with a rod shaped cell morphology that occurred in single or chains forms. The *Lactobacillus* isolate exhibited negative pattern of H<sub>2</sub>S formation and catalase activity. Sugar fermentation patterns of isolated species (Table 2) indicated that sucrose, fructose, glucose, lactose, starch and xylose were fermented by isolated *Lactobacillus* sp. The probiotic assessment test is to determine the tolerance efficiency of the isolated species to different stresses such as low pH and salinity. The results presented in Table 3 represents the survival rate of the bacterial isolate as visible growth.

### **Determination of LC50 value and assaying embryonic deformities**

The values of LC50, upper and lower confidence limits, slope function and regression coefficient results of the test sample (bacterial isolate) on the eggs at different time intervals are presented in Table 4. The ensued values (21.88, 16.21, 10.96 and 8.91 mg L<sup>-1</sup>) were calculated based on the mortality percentage (50%) for 24, 48, 72 and 96h of exposure. The 24h LC50 value of 21.88 mg L<sup>-1</sup> is approximately thrice that of 96h LC50 value of 8.91 mg L<sup>-1</sup>. Toxicity of the strain was directly proportional to the rate of mortality with a gradual increase with the increase in concentration of *Lactobacillus* isolate. The mortality data were subjected to Probit analysis and plotted against the log of dose concentration resulting in a straight line (Figure 1). During the experiment, the LC50 values reduced as the exposure time increased along with Log

concentration from 24 to 96h with no mortality in the control group. Similarly the embryo degeneration studies was performed in the eggs which were relatively grouped as control (devoid of treatment) and with treated samples (bacterial isolate and Sertraline). The untreated embryo showed an inhibition of normal development. There was a delay in the hatching time with displaying signs of micro cephalic, depigmentation and kink in tail shown in Figure 2 and these deformities were comparatively low in the *Lactobacillus* isolated sample of low concentration.

**Table 2.** Morphological, physiological and biochemical characterization of the isolated bacterial strain.

Colony Morphology	
Configuration	Round
Texture	Dry
Opacity	Opaque
Cell shape	Rod
Motility	Non motile
Pigment	White-Creamy
Gram's reaction	+
Spore(s)	-
Biochemical Tests	
H <sub>2</sub> S formation	-
Starch hydrolysis	+
Triple sugar iron	+
Gelatin hydrolysis	+
Catalase	-
Nitrate reduction	+
Acid Production from	
Lactose	+
Xylose	+
Sucrose	+
Glucose	-
Fructose	-

Legend: + Sign stands for positive and - stands for negative result

**Table 3.** Assessment of Probiotic Property.

Probiotic Assessing Parameters								
pH	2	3	4	4.5	5	5.5	6	6.5
Growth	-	++	++	++	++	++	++	+++
NaCl	2	3	4	5	6	6.5	7.0	7.5

Legend: +Tolerant -Non tolerant.

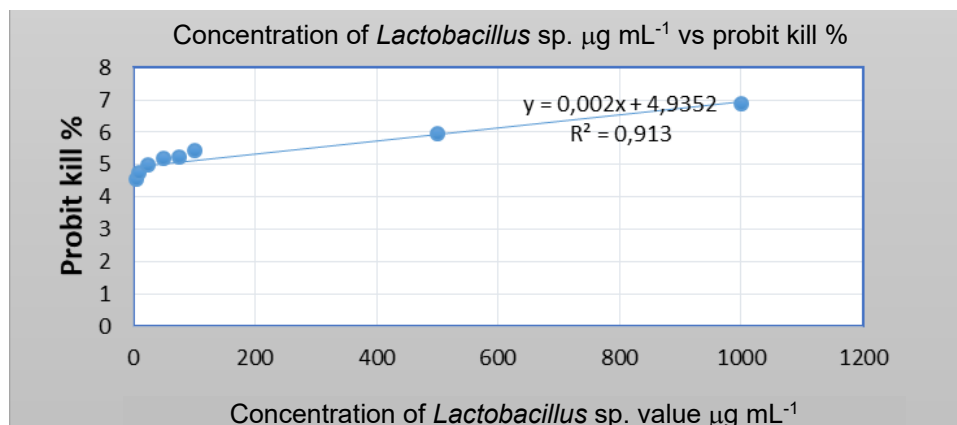
**Table 4.** Determination of LC<sub>50</sub> value.

S.No	Concentration of methyl mercury ( $\mu\text{g mL}^{-1}$ )	Log concentration	No. of embryos exposed	No. of embryos died for 96h	Probit kill %	Percent kill %
1.	5	0.698970004	30	10	4.56	33
2.	10	1	30	12	4.75	40
3.	25	1.397940009	30	15	5.00	50
4.	50	1.698970004	30	17	5.18	57
5.	75	1.875061263	30	18	5.25	60
6.	100	2	30	20	5.44	67
7.	500	2.698970004	30	25	5.95	83
8.	1000	3	30	29	6.88	97

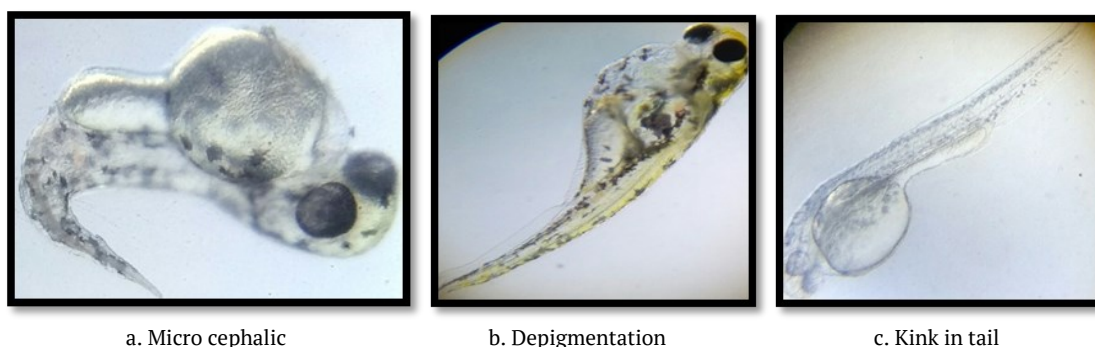
### Behavioral response –Novel tank assay

The test fish *Danio rerio* incited with lead (Pb) was exposed to the standard antidepressant drug Sertraline ( $0.1, 1 \mu\text{g mL}^{-1}$ ) and *Lactobacillus* isolate ( $50, 100 \mu\text{g mL}^{-1}$ ) to assay the behavioural pattern and is represented in Table 5. After 72h of treatment phase, the erratic behaviour of the stressed fishes exhibited altered behavioural responses. During the initial seconds of exposure time all tested concentrations showed a rapid movement, with faster opercular activity, hyper excitability and tendency of escaping from the (Pb) toxicant water. Gradually, hyper behavioural activities relatively increased and successively reduced expressing the sign of distress. Besides this, an interesting observations was that the fish had visible increase in depigmentation along with abundant mucus

secretion widely over the body with an increased exposure time. At the end of exposure (24h) period, the group without treatment struggled hard for breathing, with their reduction of swimming performance. The fish laid down on the bottom of aquaria under the exposure of heavy metal and this was assayed by novel tank method. These behavioural effects ultimately reduced in the low concentration of Sertraline and *Lactobacillus* isolate (Figure 3).



**Figure 1.** Graph of concentration *Lactobacillus* sp.  $\mu\text{g mL}^{-1}$  vs. Probit kill.



**Figure 2.** Types and frequencies of lethal effects of observed in Zebra fish embryos when exposed to methyl mercury and *lactobacillus* isolate at different time intervals: 24, 48 and 74 hours.

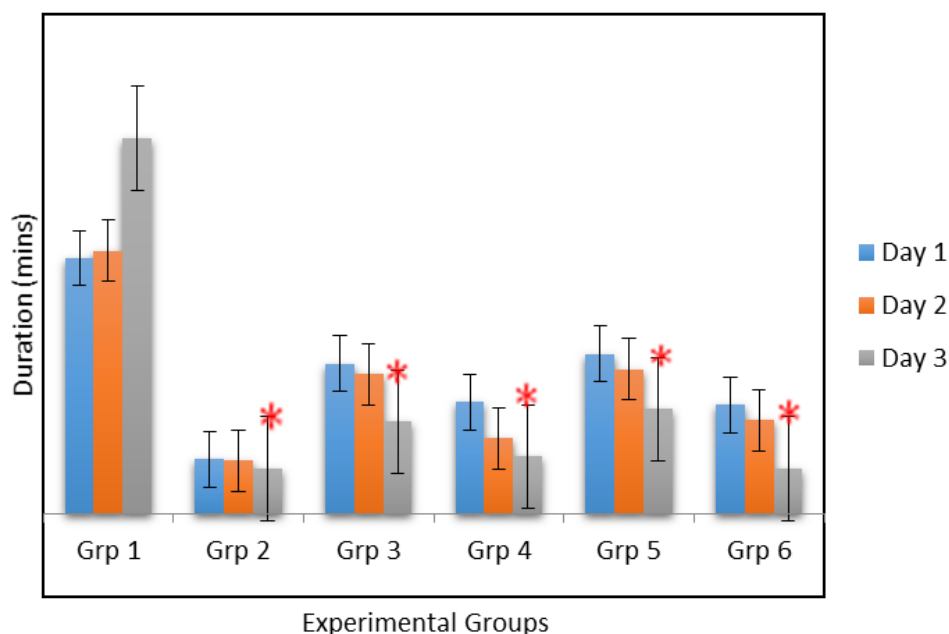
**Table 5.** The mean value of all treatments was found to be significant.

S.No	Groups	Duration of bottom dwelling(secs) /Day		
		Day 1	Day 2	Day 3
1.	1	30.67 $\pm$ 1.74*	31.50 $\pm$ 0.89*	45.00 $\pm$ 3.15*
2.	2	6.50 $\pm$ 0.77	6.33 $\pm$ 0.76	5.33 $\pm$ 0.56
3.	3	18.00 $\pm$ 0.58*	18.67 $\pm$ 0.88*	11.00 $\pm$ 1.32*
4.	4	13.33 $\pm$ 1.05*	9.00 $\pm$ 0.58*	6.80 $\pm$ 0.60*
5.	5	19.17 $\pm$ 0.70*	17.83 $\pm$ 0.30*	12.50 $\pm$ 0.76*
6.	6	13.00 $\pm$ 1.46*	11.17 $\pm$ 0.48*	5.33 $\pm$ 0.67*

\*Values represented mean  $\pm$  S.E.M. (n = 6), \*p < 0.05 vs. control (group 1).

### Lymphocyte counting assay

Lymphocytes contained a small amount of blue cytoplasm containing granules, with round nuclei that could be indented and, in contrast to thrombocytes, displayed a stippled or smudged chromatin (Figure 4). Large mononuclear cells were evident that are reputed as large lymphocytes containing a moderate amount of dark blue cytoplasm, often with vacuoles and a round or irregular nucleus. Small lymphocytes contained similarly dark stained nucleus but with less cytoplasm. Comparing the percentages of lymphocytes with the control and normal a slightly higher percentage was found in *Lactobacillus* high concentration however this lead to aggregation. The percentage of lymphocytes in *Lactobacillus* low concentration and sertraline high concentration were relatively equal with an increased significant compared to the toxic sample (Table 6) but devoid of cell aggregation. Hence at this concentration lymphocyte proliferation occurred in a controlled manner.



**Figure 3.** Effect of test samples in treatment phase (Day 1 -3) in assaying the Bottom dwelling duration.

(\*) indicate statistical significance (n = 6), with p-value thresholds of < 0.05 vs. control (group 1).

**Table 6.** The percentage of counted lymphocyte.

S. No	Groups	Treatment	Counted lymphocyte in %
1.	I	Devoid of treatment (with Pb)	40
2.	II	Normal	65
3.	III	Pb+ strain (high)	60
4.	IV	Pb + strain (low)	65
5.	IV	Pb + setraline (high)	56
6.	V	Pb+ setraline (low)	47

### Histological analysis

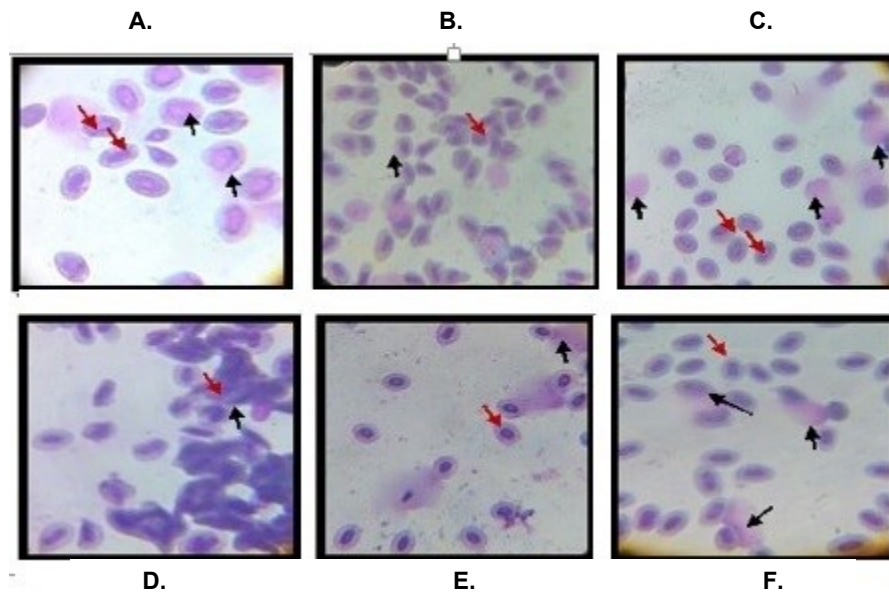
The histological slides of the treated samples in comparison with normal and non-exhibit the presence or the absence of any alteration such as presence of macro vacuoles, degeneration and necrosis, inflammatory infiltration, haemorrhage, and adiposities. The general histological screening indicated a low to high incidence of damage on the cerebellum of Zebra fish induced with lead and treated test samples. The control group did not show any deformities but the toxic induced samples had varied differences in the structures of the brain. As shown in Figure 5 high degree of necrosis (loss of cell) accompanied by infiltration of haemocytes and gliosis is observed with wide intercellular spaces. A huge loss of purkinjee cells was observed in the (Pb) intoxicated fish and this has been restored in the *lactobacillus* low concentration treatment.

### Discussion

This study intends to evaluate the neuroprotective effect of bacterial isolate from fermented milk by targeting neurotoxicity and regulating immune response. The bacterial cultures isolated were identified according to their physiological and biochemical characterization. All isolates were gram positive, rod shaped, non-spore forming, non-motile and catalase negative. The bacterial isolate was identified considering these features as common characters of *Lactobacillus* species which also coincided with the results of Patil, Pal, Anand, and Ramana (2010) and Forouhandeh et al. (2010). Instigating a bacteria as a probiotic requires a stringent endurance against stressful environment in the gastro intestinal tract such as acidity and tolerance Arshad, Mehmood, Hussain, Annus Khan, and Khan (2018) In particular a probiotic in stomach has to endure low pH (3.0) for long hours before it reaches to the lower part of digestive tract (Saarela, Mogensen, Fondén, Mättö, & Mattila-Sandholm, 2000). The growth of bacterial isolate interpreted the resistance of probiotic against stressful conditions (Ouweland, Kirjavainen, Shortt, & Salminen, 1999).

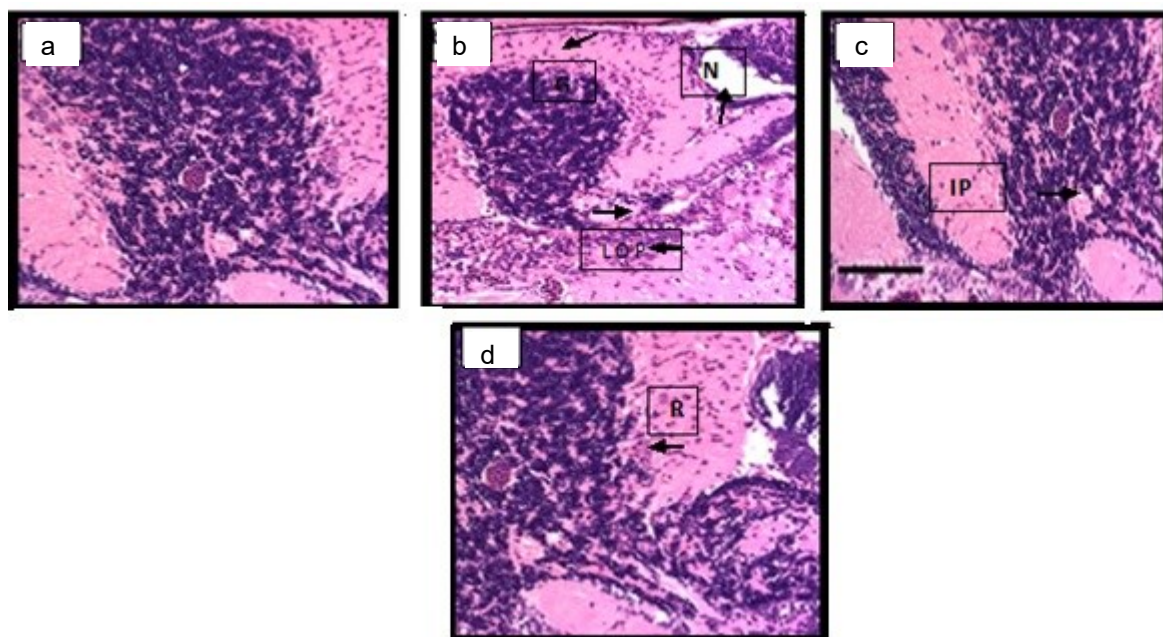


Relative extent of visible growth was more in pH 6.5 which was pursued by pH 3. Comparably, the percentage of salinity revealed endurable growth from 2 - 6.5% of NaCl concentration. Thus these experimental assays ensure the consideration of the isolated bacterium as a probiotic formulation.



**Figure 4.** Zebrafish hematology. Wright/Giemsa stained zebrafish blood smears (A-F)\* Differentiating lymphocytes (black arrows) from thrombocytes (red arrows) in the respective groups. Blood smear images were captured under 100x total magnification with oil.

\*A-Normal; B-Toxin induced (Pb); C- Pb+ Strain (low); D- Pb + Strain (high); E- Pb + Setraline (low); F- Pb + Setraline (high).



**Figure 5.** Histopathological changes in cerebellum regions of the a.normal b. Pb incited and un treated groups c. Pb + Setraline (low) d. Pb+ Strain (low) (resolution 400x).

Note: G- Gliosis ; N- Necrosis ; IP- Inflammation infiltration on hepatic parenchyma; LOP- Loss of Purkinje R- Restoration of Purkinjee).

Acute toxicity of a restorative formulation is generally evaluated by the lethal dose value which is an indication of the lethal toxicity of a given substance. Toxicity test was performed to determine the embryonic deformities at 24, 48, 72 and 96h. LC50 values using eight concentrations of (5, 10, 25, 50, 75, 100, 500 and 1000 ug) *Lactobacillus* species was incited on the eggs of zebra fish. The data obtained from the toxicity test revealed that the mortality of eggs increased with increasing concentration and exposure time. Similar to the predicted LC50 value of the strain it is also significant to know the value of heavy metal used and it was refer from previously reported studies (Batool & Javed, 2015; Chinni & Yallapragada, 2000; Ferrer, Andrade, Asteasuain, & Marcovecchio, 2006; Singh & Ansari, 2017; Ullah et al., 2016). The



contamination of heavy metals in water and sediment, when occurring in higher concentrations, is a serious threat because of their extended persistence, and bioaccumulation which can intervene the food chain. Lead is a naturally occurring heavy metal characterized as toxic substance. Exposure to high lead levels in the aquatic system can cause physiological impairment and genomic alteration in fish and other aquatic organisms. This has the definite impact on human and hence research related to lead toxicity is highly significant and demanded as a prerequisite.

The adult fishes were experimented to do a behavioural assay test (novel tank method) that enumerates the intensity of stress induced by the neurotoxin. During the initial moments hyper excitability and tendency of escaping from the toxicant water was observed. Later on these hyper behavioral activities relatively increased and successively reduced expressing the sign of distress which correlated with the findings of Levin et al. (2007). This is a widely used method to assess the consequences of stressful manipulations in Zebra fish. It was explicitly introduced as an assay for anxiety by Levin et al. (2007) who reported that adult Zebra fish spend about 50% of a 5 min. session in the bottom of a novel tank ('bottom-dwelling'), and thus antidepressant drugs decreases this preference. The percentage of lymphocytes were similar both in low concentration of bacterial isolate and high sertraline and this exceeded the count in the toxic sample but devoid of cell aggregation. Hence, low concentration of the probiotic isolate determined the lymphocyte proliferation in a regulated manner. Hematology of Zebra fish complements the growth of molecular tools for marking immune cells. Hematological findings of zebra fish reports that average percentage of peripheral blood differential is 71-92% (Murtha, Qi, & Keller, 2003).

This study, provides a range of 47-65% where, the highest was seen in the normal groups persuaded by the *Lactobacillus* high concentration. However all the treated groups showed an increase count in the lymphocyte when compared to the lead induced sample. This suggests that the injection of bacterial isolate and sertraline had some immunostimulatory effect, possibly through the external mucous layer into the body cavity. These expanded percentages could represent large lymphocytes plasma blasts with expanded Golgi bodies (Bennett et al., 2001). The shift toward higher percentages of lymphocytes corresponds to an immunomodulatory effect on the toxic heavy metal. Histopathology has been widely used as biomarker in assessing the health of toxin exposed brain cells. This study showed that brain cells are severely damaged when exposed to lead when compared to control groups. Conversely, the treated groups with low dosage of *lactobacillus* isolate and Sertraline restored necrosis to certain extent.

## Conclusion

Gut microbe is integral to various metabolic processes that promote health. In particular during pregnancy the proliferation of these microbes patrol the growing fetus from the influence of prenatal stress. Lead a global pollutant and is considered as a neurotoxin that interrupts neuroprotection that can cause developmental neural deformities. The consumption of synthetic neurodrugs can appease the intensity of mental ailment but also elevates the occurrence of adverse effects. Further neurotoxicity, activates the peripheral immune system by stimulating inflammation through pro-inflammatory mediators. This weakens the maternal immunity thereby affecting the vitality of the baby. Gut microbes, whose pathology can disrupt gut epithelial wall and release into the systemic circulation, are found to interact and regulate immune response. This study initiates a balance between the effects of neurotoxic and neuroprotective intensity in the brain which is obtained by controlling excess neuroinflammation.

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